ECTOPIC BMP EXPRESSION DISRUPTS SKELETAL DEVELOPMENT IN THE JAW OF AVIAN EMBRYOS

Introduction:
Development of the skeleton occurs through a multitude of processes. In most of the body, the majority of cartilages that form are templates for development of the bony skeleton. These skeletal elements are initially composed of cartilage which is replaced by bone through the process of endochondral ossification. In the skull, in addition to endochondral ossification, bones form directly through the process of intramembranous ossification. Further, some cartilages in the skull do not undergo hypertrophy, are not replaced by bone, and remain as persistent cartilage. These different modes of skeletal formation suggest that regulation of cell differentiation and maturation may be distinct in the head and trunk. As a step toward understanding mechanisms underlying development of intramembranous bone and persistent cartilage, we examined the effects of exogenous Bone morphogenetic proteins (Bmps) on development of the skeleton of the face since these molecules induce regulators of chondrocyte and osteoblast differentiation.

Materials and Methods:
Avian embryos were incubated to stage 22 [1], a small hole was made in the shell, and a replication competent retrovirus (RCAS) encoding Bmp2, Bmp4, or, as a control, alkaline phosphatase was injected into the mesenchyme of the mandible. Embryos were incubated for 24, 48, or 72 hours and for 14 days and for 14 days of incubation, fixed in 4% paraformaldehyde. Embryos were photographed, and either dehydrated, embedded in paraffin and sectioned (10μm), or stained with alcian blue/alizarin red. Bmp expression was not observed. At this time a protuberance was observed within the jaw and this was accompanied by an increase in cartilage and muscle (not shown) and altered the patterns of bone (Fig. 1) that were present within the jaw.

Bone morphogenetic proteins (Bmps) on development of the facial skeleton, we mis-expressed Bmp2 and Bmp4 in the developing jaw beginning at HH 22. Within 72 hours of infection phenotypic variations in treated embryos were observed. At this time a protuberance was observed within the jaw and this was accompanied by an increase in cartilage within the mandible and tongue (Fig. 1). In situ hybridization revealed that the retroviral vector was distributed widely throughout the mesenchyme of the jaw (Fig. 2). By 15 days of development, the developing mandible was dysmorphic and a large amount of cartilage was present in the lower jaw (Fig 1). This cartilage did not exhibit any characteristics of hypertrophic cartilage. Treatment with Bmps 2 and 4 also led to a decrease in the amount of muscle (not shown) and altered the patterns of bone (Fig. 1) that were present within the jaw.

The increased cartilage and decreased bone formation was preceded by molecular alterations. Sox9 expression was not down-regulated in neural crest mesenchyme (Fig. 3), however Runx2 expression was reduced in the mesenchyme of treated mandibles (Fig. 2).

Discussion:
Ectopic Bmps lead to increased chondrogenesis in neural crest-derived skeletal progenitor cells. A similar observation has been reported after ectopic expression of Bmps in developing limb buds [2]. Our results also indicate that during development of the mandible, sustained high levels of Bmp2 or Bmp4 alters bone formation by changing the domain of Runx2. Thus the differences in skeletal phenotypes such as endochondral vs. intramembranous ossification and persistent versus replacement cartilage do not appear to reside in the intrinsic response of cells to Bmp signals.

Fig. 1. Jaw disruptions following Bmp mis-expression.
(A) A control embryo at 14 days of incubation illustrates the normal skeletal pattern in the lower jaw. (B) After Bmp mis-expression, a large amount of cartilage is present in the mandible and the amount of bone appears reduced.

Fig. 2. Molecular affects of ectopic Bmp.
(A) vEnv is not present in normal embryos, but (B) is widespread after infection. (C) Sox9 is restricted to the mandibular cartilage (ct), but (D) is expressed (E) Runx2 is expressed adjacent to the cartilage, but (F) is diffuse in treated embryos.

Bibliography:

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